
DANGER SIGNALS FROM ALLERGENS: PROTEOLYTIC ACTION

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What makes an allergen an allergen?

What is an allergen?

- a molecule inducing (specific) IgE antibodies

and/or

- a molecule triggering a clinical allergic reaction

A protein that can do both is a “complete” allergen

Is there a link between structure/function and allergenicity?

Editors' choice articles

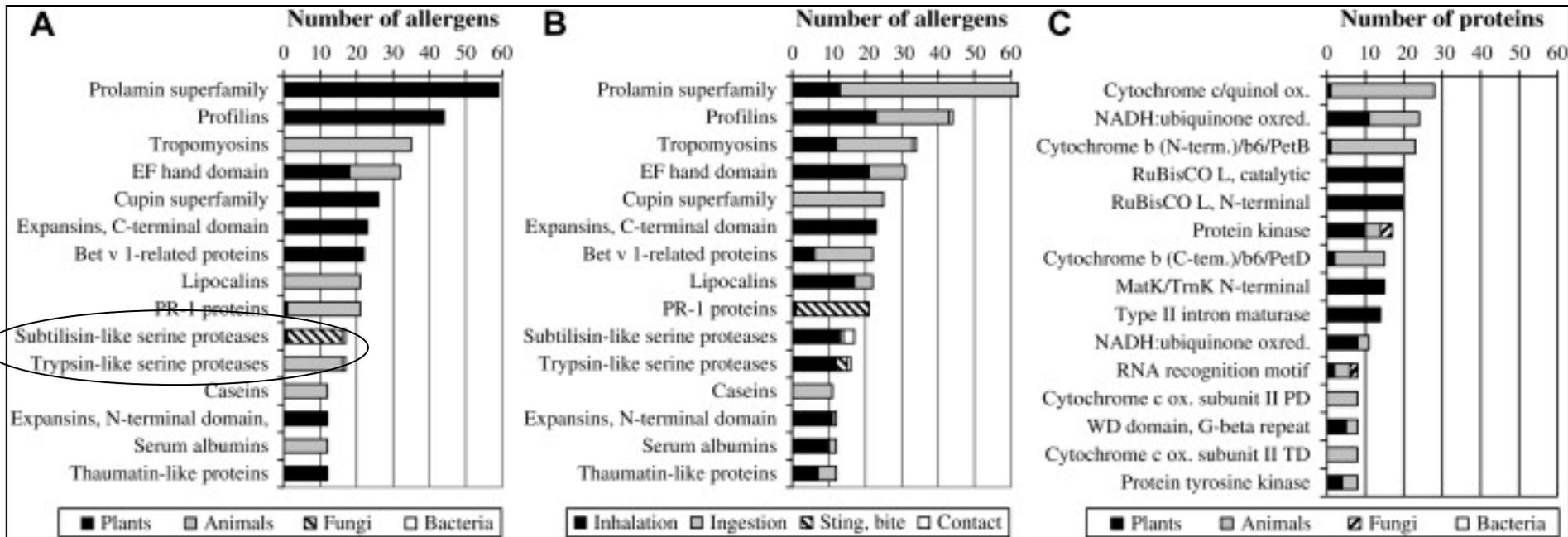
Allergens are distributed into few protein families and possess a restricted number of biochemical functions

Christian Radauer, PhD,^a Merima Bublin, PhD,^a Stefan Wagner, PhD,^a Adriano Mari, MD,^{b,c} and Heimo Breiteneder, PhD^a *Vienna, Austria, and Latina and Rome, Italy*

Conclusion: The small number of protein families that contain allergens and the narrow functional distribution of most allergens confirm the existence of yet unknown factors that render proteins allergenic. (*J Allergy Clin Immunol* 2008;121:847-52.)

Clinical implications: The classification of allergens supports the elucidation of factors that make proteins allergenic, thus possibly paving the way for novel therapeutic concepts.

Basis for claim that structure/function predicts allergenicity:



Indeed, most allergens identified so far belong to a limited number of protein families. Is this really structure and/or function driven??

Cross-reactivity and “fashion” are a good basis to boost one’s PubMed score but of course it does bias the list of known allergens

Having said that, there is probably sufficient evidence to support a role for structure/function of molecules as determinants of allergenicity.

What makes an allergen an allergen?

Here we will mainly focus on the capacity to sensitise, i.e. on “complete” allergens.

Complex issue:

- *intrinsic factors*, i.e. molecular properties
 - such as:
 - proteolytic activity
 - lipid-binding
 - protease resistance
 - heat resistance
 - affinity for PRR
- *extrinsic factors*, i.e. anything “accompanying” the molecule
 - such as:
 - matrix presenting the molecule
 - co-exposures in environment
 - infections
 - microbiome
 - degree of exposure
 - genetic background
 - timing and frequency of exposure

So,

It certainly is no simple straightforward story to predict allergenicity:

- genetic predisposition
 - gene-environment interactions
 - complex environmental exposures
 - diet in the broadest sense
 - timing of exposure
 - hygiene
 - lifestyle
 - vaccinations
 - migration
 -
 -
-

Intrinsic pro-allergenic properties can not be more than one of a multitude of factors that in the end determine whether somebody develops an allergy against a protein.

Not everybody exposed to a protein with pro-allergenic properties will develop IgE antibodies against it, let alone develop a clinical allergy.

Even if family background, westernized sedentary lifestyle, and early exposure to protein with reported pro-allergenic properties (e.g. Der p 2) come together, the exposed subject can stay healthy or become allergic to pollen instead of mite.

Enough relativization, what is the evidence supporting a pro-allergenic role of proteolytic action.

Several major allergens have been classified as proteases:

- Group 1 house dust mite allergens are cysteine proteases (e.g. Der p 1 and Der f 1)
- Group 3, 6, 9 house dust mite allergens are serine proteases
- Cockroach aspartic proteinases (e.g. Bla g 2)
- Mould serine proteases
- Pollen proteases?

So what are the postulated mechanisms by which proteases facilitate induction of sensitization and allergic symptoms?

Most data in support of proteolytic action as an answer to the question “what makes an allergen an allergen?” come from studies on the group 1 house dust mite allergens Der p and f 1.

Three main targets of Der p 1/Der f 1's protease activity:

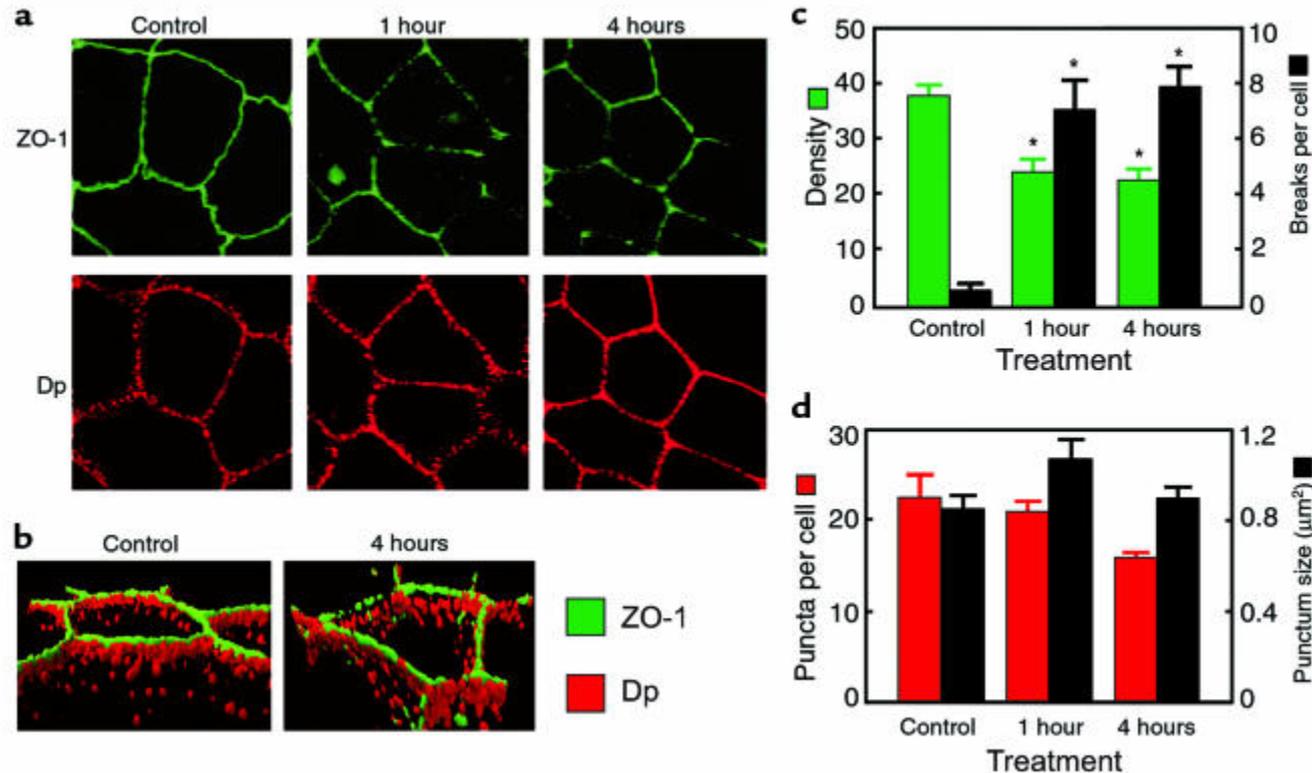
- physical barrier function
 - innate response of structural cells
(epithelial cells / keratinocytes)
 - adaptive immune response
-

Physical barrier function

Der p 1 facilitates transepithelial allergen delivery by disruption of tight junctions.

Wan H, Winton HL, Soeller C, Tovey ER, Gruenert DC, Thompson PJ, Stewart GA, Taylor GW,
Garrod DR, Cannell MB, Robinson C.

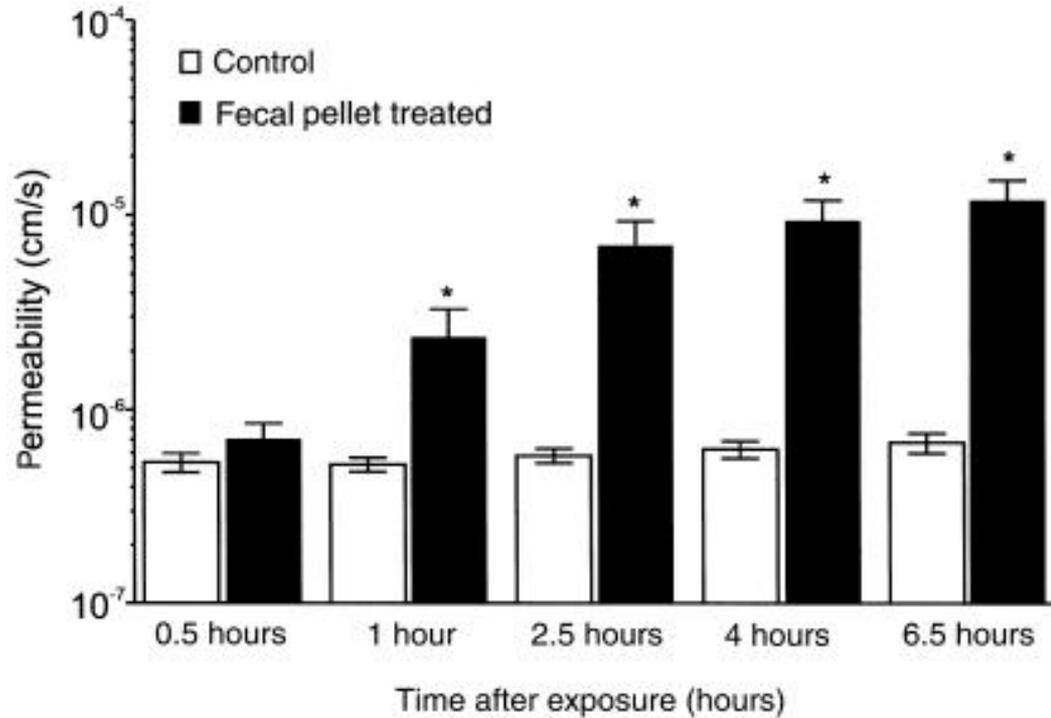
J Clin Invest 1999; 104:123-133



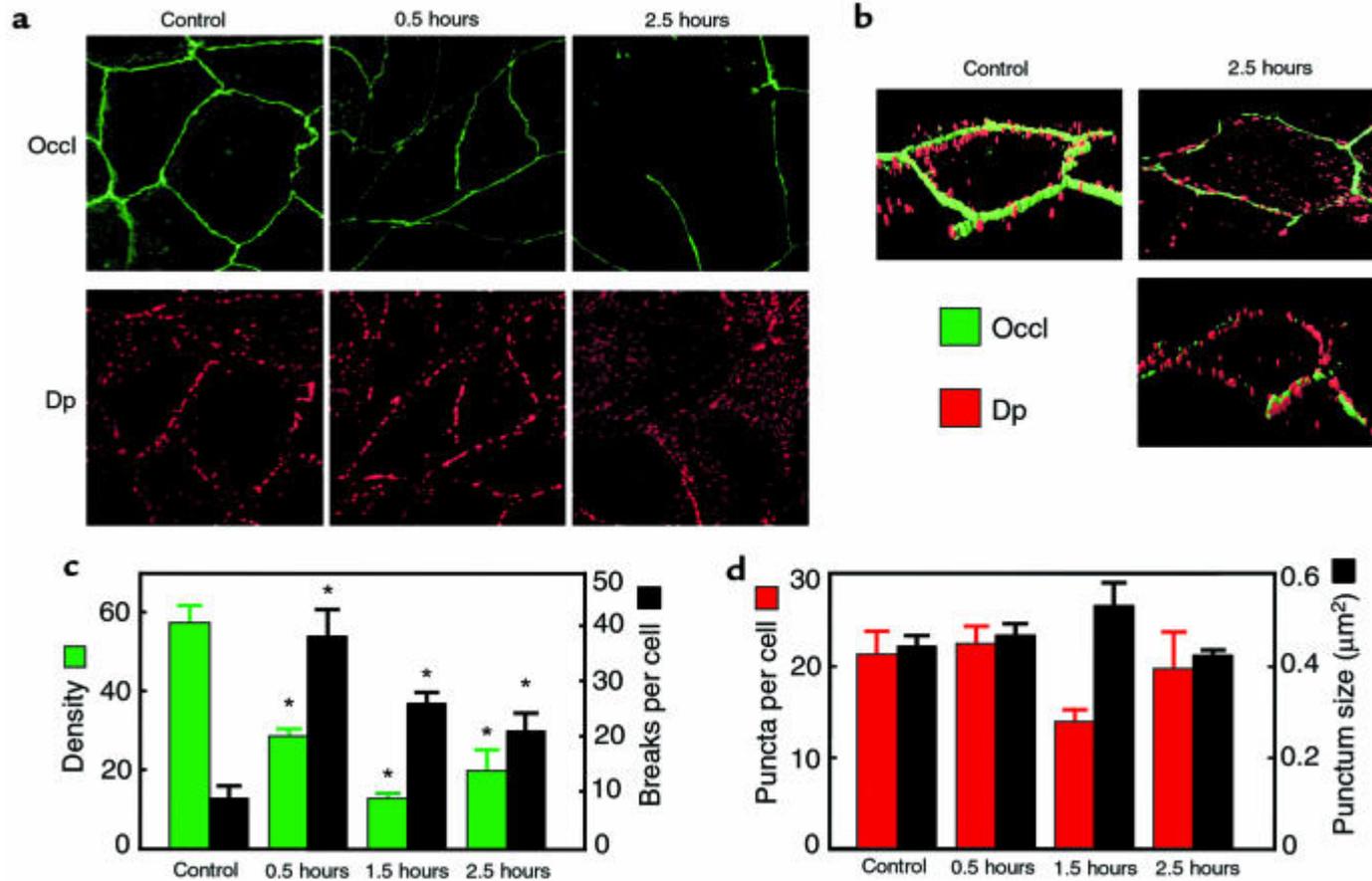
ZO-1 and Dp (desmoplakin) are tight-junction proteins.

Breakage upon fecal particle exposure is clear for ZO-1 (in green)

Increased permeability of epithelial monolayer in transwell system using radiolabeled (^{14}C) mannitol.

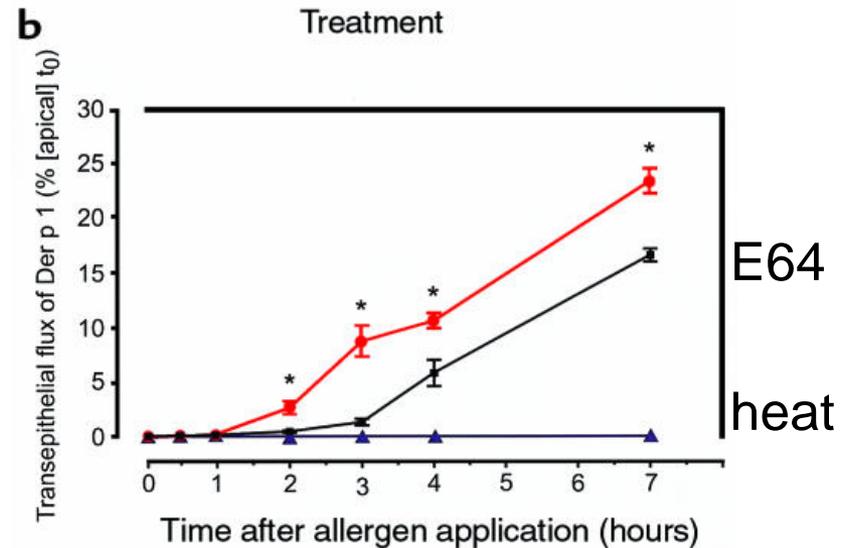
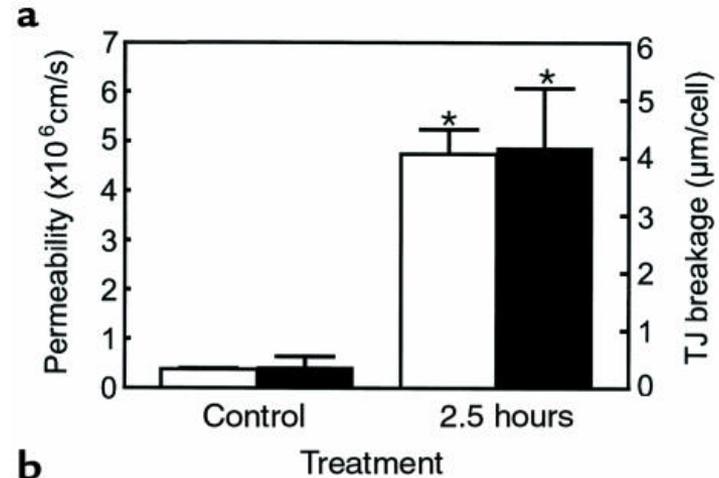


Effect of purified Der p 1 on tight junctions: occludin staining



TJ breaks and increased transport of mannitol go hand in hand.

A specific cysteine protease inhibitor (E64) and heat treatment prove that proteolytic action is instrumental in the increased permeability.



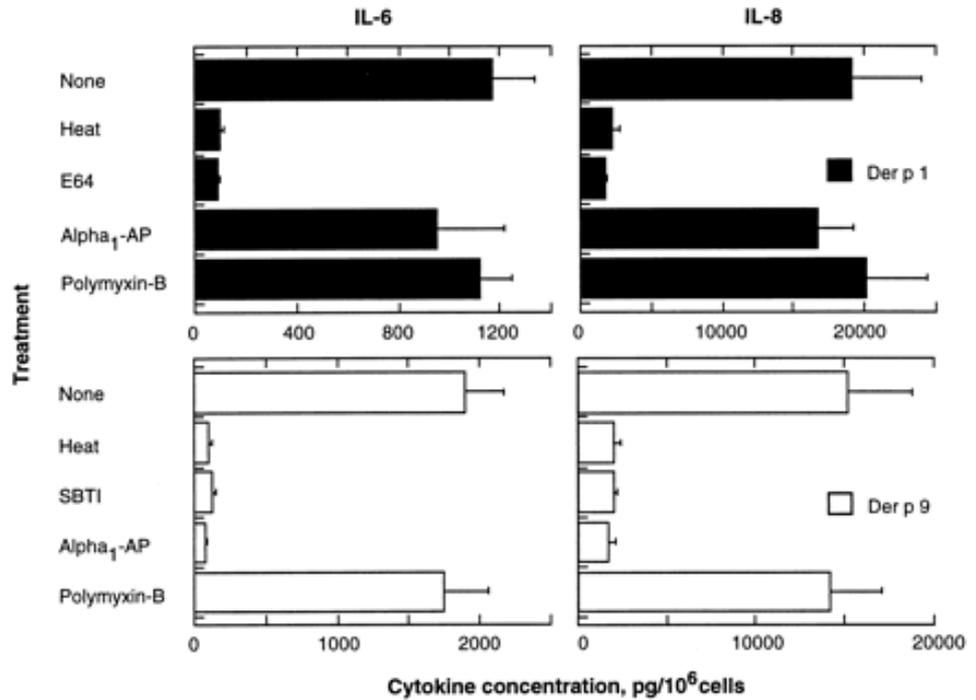
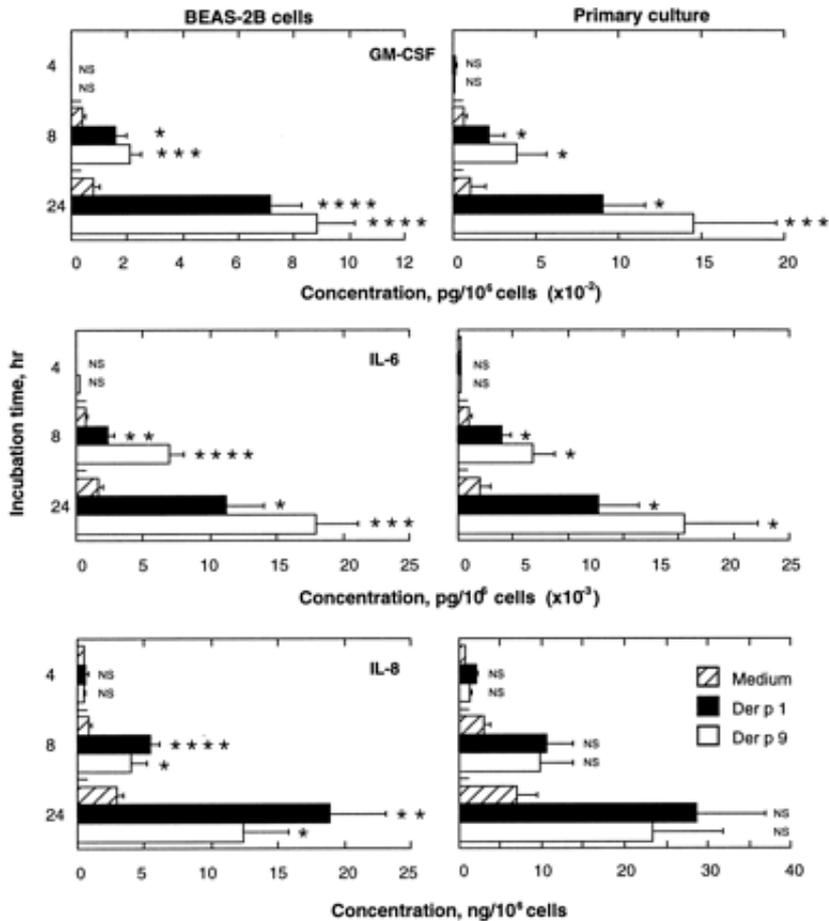
Barrier function is affected by proteolytic action in vitro

Innate responses of structural cells

Dust mite proteolytic allergens induce cytokine release from cultured airway epithelium.

King C, Brennan S, Thompson PJ, Stewart GA.

J Immunol 1998;161: 3645-3651



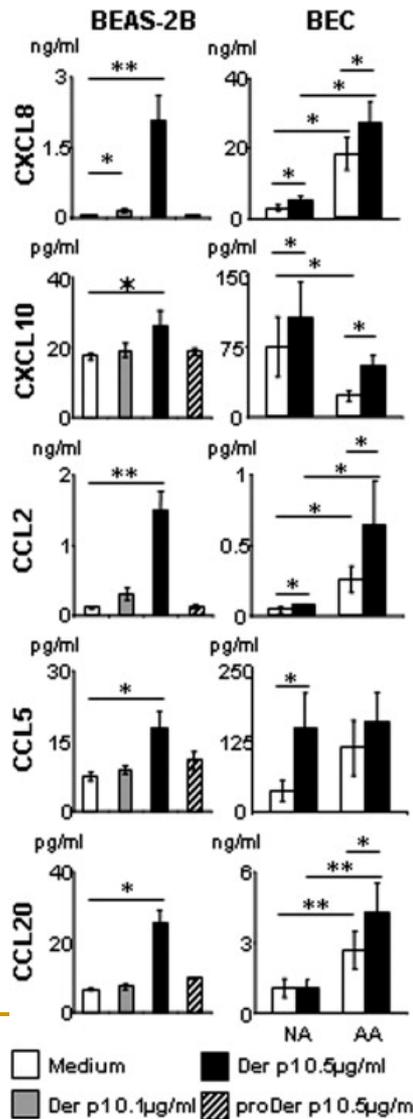
Induction of pro-inflammatory cytokines by Der p 1 (and 9)

Dependency on proteolytic action of Der p 1 (and 9)

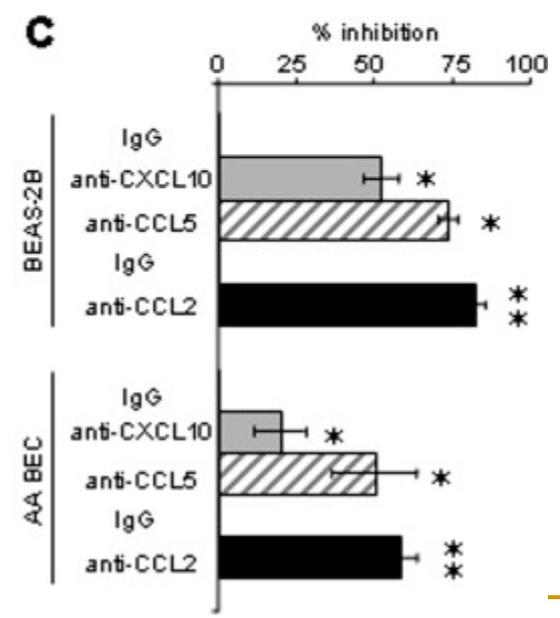
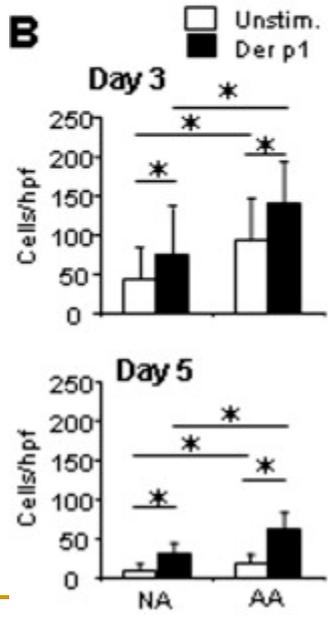
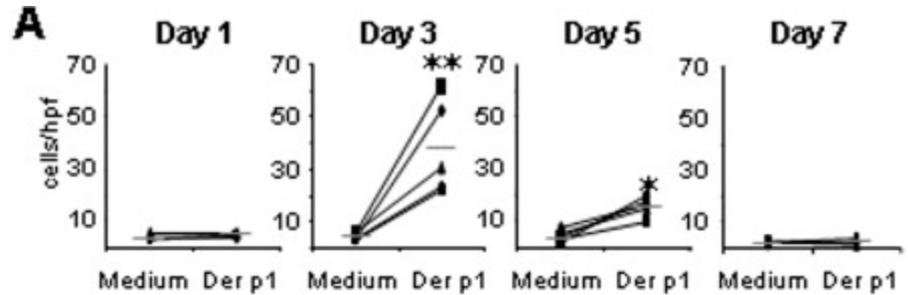
Asthmatic bronchial epithelium activated by the proteolytic allergen Der p 1 increases selective dendritic cell recruitment.

Pichavant M, Charbonnier AS, Taront S, Brichet A, Wallaert B, Pestel J, Tonnel AB, Gosset P. J Allergy Clin Immunol 2005; 115: 771-778

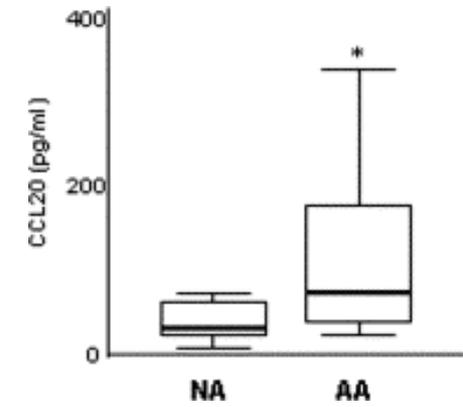
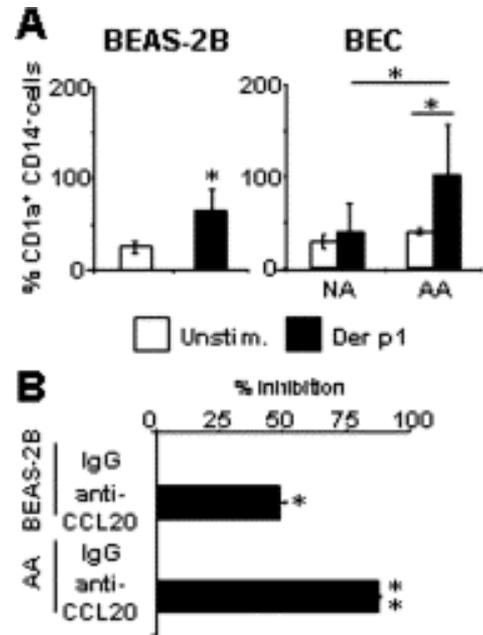
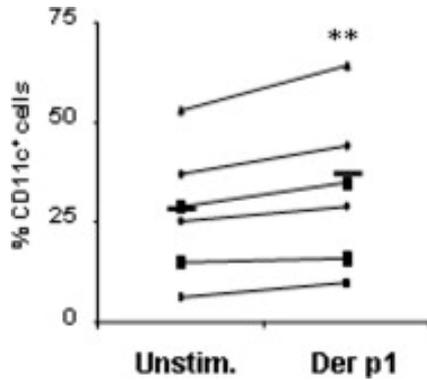
Chemokine production induced by Der p 1



DC recruitment upon Der p 1 exposure



Dependency on chemokines



Migration of DC into the epithelium

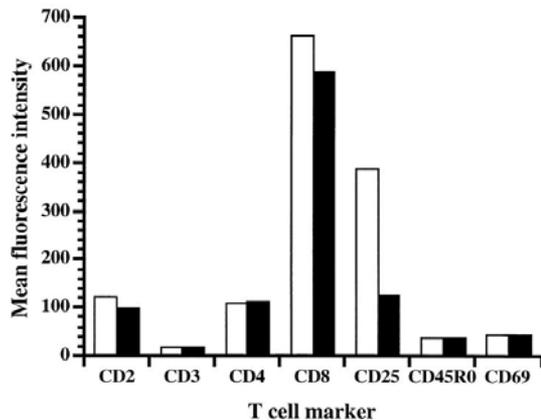
Effect is more pronounced in allergic asthmatics (AA)

Proteolytic action triggers structural cells to release inflammatory cytokines and chemokines, leading to influx of DCs, again in vitro

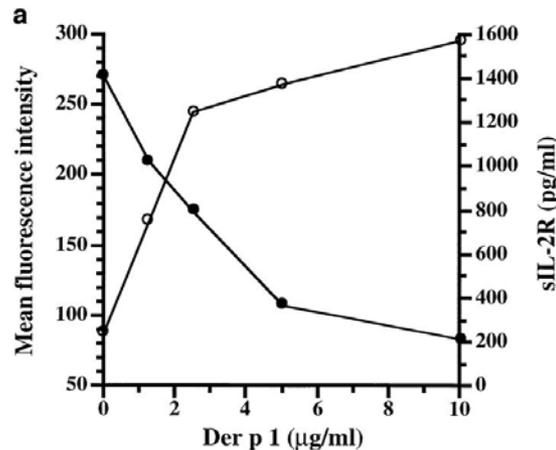
Adaptive immune response

Proteolytic cleavage of CD25, the alpha subunit of the human T cell interleukin 2 receptor, by Der p 1, a major mite allergen with cysteine protease activity.

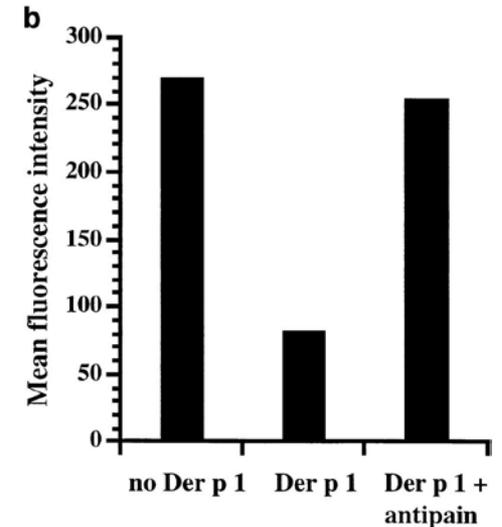
Schulz O, Sewell HF, Shakib F.
J Exp Med 1998; 187: 271-275



Cleavage of CD25



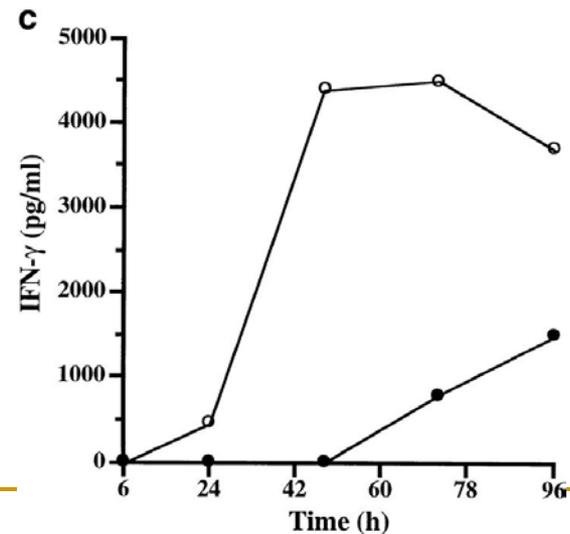
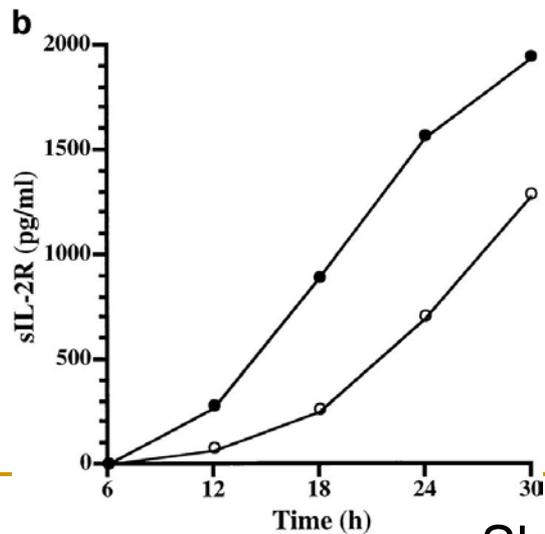
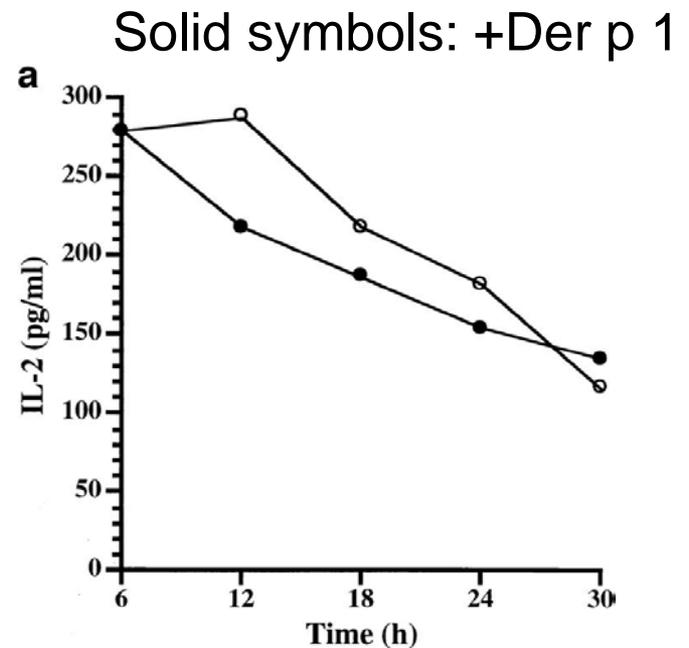
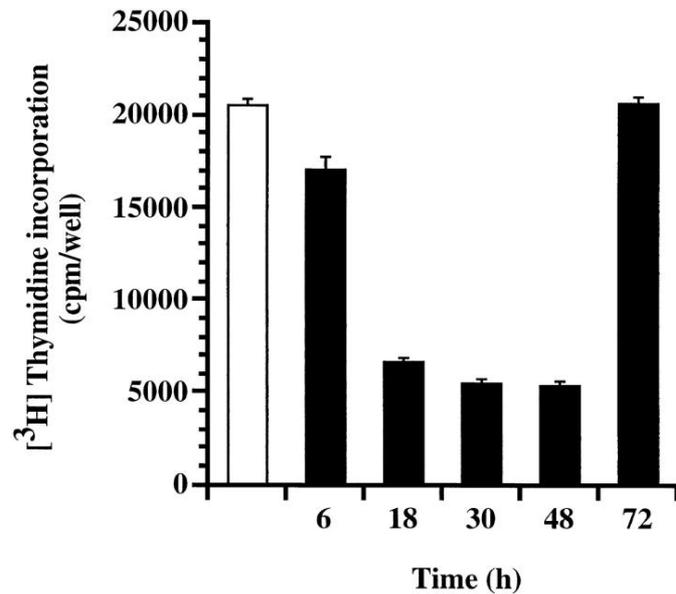
Increase soluble CD25



Blocked by specific inhibitor

Overall effect is postulated to be inhibition of Th1 thereby favoring Th2

Inhibition aCD3-induced T-cell proliferation in which CD25 is critical



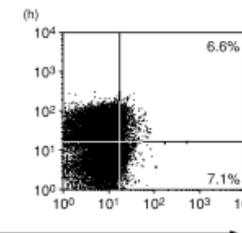
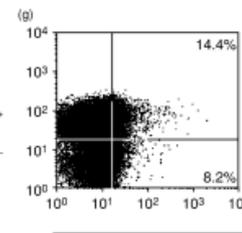
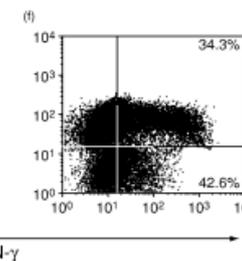
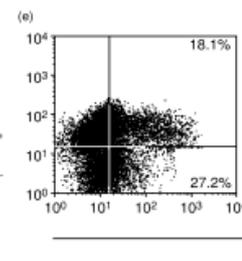
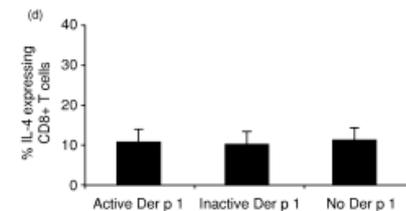
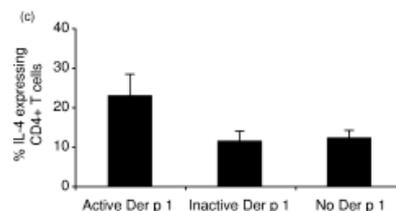
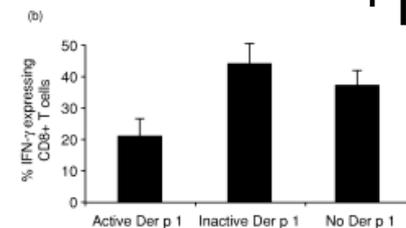
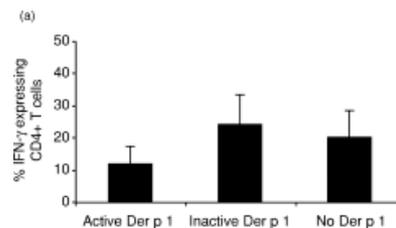
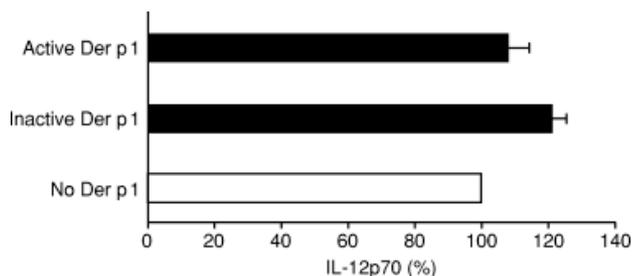
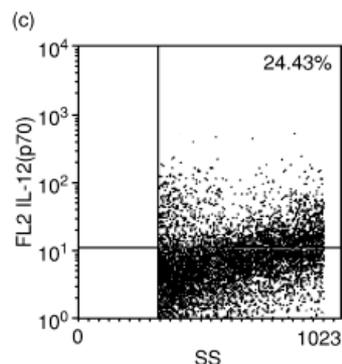
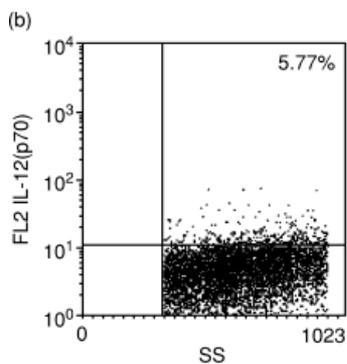
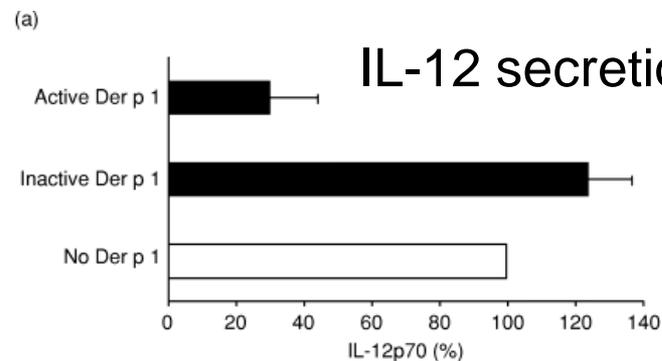
Skewing away from Th1

The proteolytic activity of the major dust mite allergen Der p 1 conditions dendritic cells to produce less interleukin-12: allergen-induced Th2 bias determined at the dendritic cell level.

Ghaemmaghami AM, Gough L, Sewell HF, Shakib F.

Clin Exp Allergy 2002; 32: 1468-1475

IL-4/IFN γ \uparrow



Intracellular IL-12 is unaffected

active Der p 1

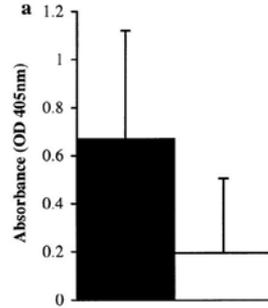
inactive Der p 1

The cysteine protease activity of the major dust mite allergen Der p 1 selectively enhances the immunoglobulin E antibody response.

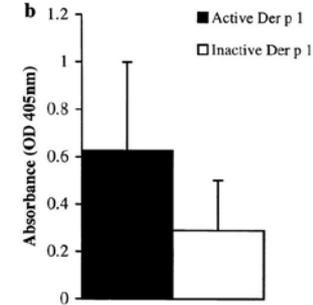
Gough L, Schulz O, Sewell HF, Shakib F.

J Exp Med 1999; 190:1897-1901

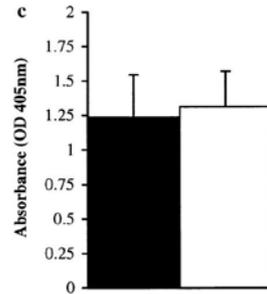
Total IgE



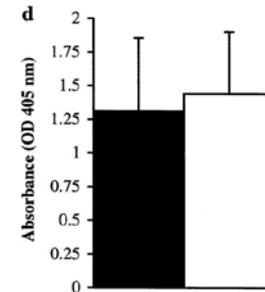
Specific IgE



IgG



IgG1

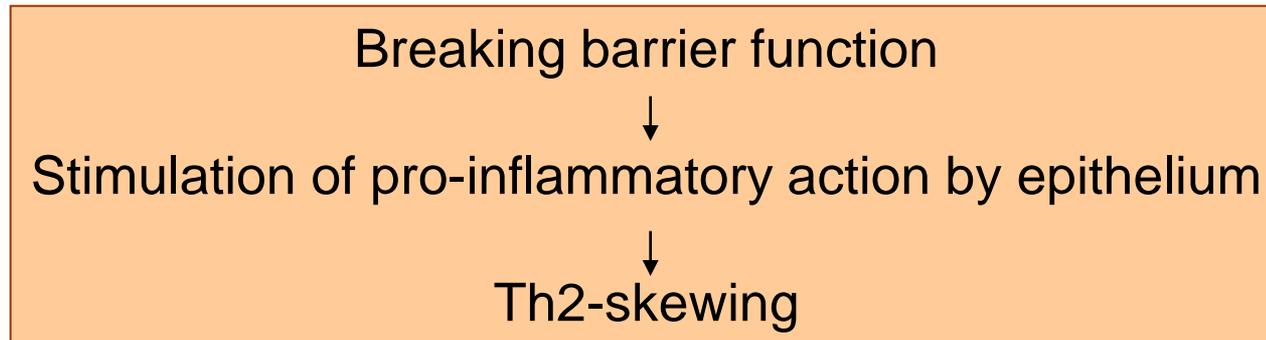


IgE induction requires proteolytic action as illustrated by addition of E64

Proteolytic action favours skewing towards Th2

Concluding remarks

There is certainly quite some evidence from *ex vivo*, *in vitro* and mouse model experiments that there is a potential pro-allergenic pathway triggered off by proteolytic action.



Whether this is also the case *in vivo* in human subjects is hard to say.

Little data available about the actual proteolytic activity of house dust, but it certainly is far more complex than just mite proteases.
